

Using Caged Mussels to Characterize Exposure from Point and Nonpoint Stressors in the Cherry Point Reach: The Value of *In situ* Monitoring

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Extended Abstract

Caged mussel studies were conducted during the spring spawning season of 1998, 1999, and 2000 along the Cherry Point Reach (CPR) on the northwestern coast of Washington state for the Department of Natural Resources. The 2000 “programmatic study” included three other geographical areas in Puget Sound for comparative purposes, Fidalgo Bay (FB), Port Gamble (PG), and Brownsville (BV). The purpose of the most recent study was to facilitate comparisons of polycyclic aromatic hydrocarbon (PAH) exposures and associated effects along the CPR with conditions at other herring spawning sites. These comparisons are important because the CPR has historically been the most prolific herring spawning grounds and because herring populations there have declined more drastically than in other areas. Comparing conditions in other spawning areas with the relative rates of decline could provide additional insight as to the generic causes for the overall decline or site-specific causes associated with declines in particular geographic areas. Bioaccumulation of PAHs in mussel tissues was used as an indicator of herring egg exposure, mussel growth rates were used as an indicator of potential effects, and PAH tissue burdens in mussels were compared with effects data from other studies using other species. Water temperature was measured with *in-situ* monitors at 15-minute intervals to help determine the potential effects of temperature that was identified as a stressor to herring eggs in the 1998 and 1999 Cherry Point Studies.

These studies were designed to answer or provide insight into three key issues:

- What is the potential PAH exposure to herring eggs in these geographical areas?
- Are there any effects of PAH exposure on mussel growth?
- Is temperature another potential stressor for herring eggs?

Each year approximately 2000 mussels (*Mytilus galloprovincialis*) were collected from the Taylor United mussel farm, sorted into size groups, assigned to cages, and transplanted to multiple sites in approximately 18 feet of water at each test area for approximately 60 days. Since mussel size has a significant effect on bioaccumulation and growth, the size range was limited to approximately 10 mm to minimize variability in measurement endpoints. Compartmentalized cages were used to facilitate repeated measurements on the same individual mussels at the beginning and end of the test. Multiple measurements for whole-animal weights and shell lengths on the same individual mussels improved the confidence in the measurement data, and increased the discriminating power of the test for evaluating growth and detecting differences in effects among sites or geographical areas. Growth of individual mussel shells and tissues were also used to characterize biological effects associated with exposure to PAHs.

Along the CPR in 1998 there were 12 sites with two cages each (90 mussels/cage), and in 1999 there were 44 sites with one cage each (51 mussels/cage). In 2000, three cages with 48 mussels each were deployed at each site: five sites were monitored along the CPR, and three sites were monitored at each of the other three areas; FB, PG, and BV. Mussel cages were retrieved after a 60-day exposure period, but only 60% were retrieved in 2000 because of an experimental deployment and retrieval system that was less successful than in previous years (i.e., retrieval success was 100% in 1998, and 93% in 1999). Mussel growth was measured after retrieval. Mussel tissues were retained for chemical analysis. Statistical analysis of exposure and effects data demonstrated that there significant differences among geographic areas in the 2000 study. Bioaccumulation of PAHs in soft tissues from live mussels was used to characterize potential chemical exposure at each of the four geographic areas. Pooled tissue samples were prepared by combining the soft tissues for all living mussels from a given cage. In the 2000 study, this provided between one and three tissue samples per site, depending on the number of cages retrieved at the end of the test.

To estimate initial tissue weights and establish a baseline concentration of PAHs in mussel tissues before deployment each year, tissues from approximately 200 mussels in the identical size range as those deployed were weighed at the beginning of the test and stored for chemical analysis. At the beginning of any test, there was no statistically significant difference in the size of the mussels (i.e., shell length and whole-animal weight) among cages, sites, or geographical areas, including mussels used for the baseline measurements. These initial measurements were used to estimate changes in tissue and shell weights by comparing them with measurements on individual mussels at the end of the test.

Average mussel survival was 47% in 1998, 57% in 1999 and 95% in 2000 over the 60-d exposure period. Mussel growth was similar for most mussel metrics along the CPR in all three years, but significant differences in growth were found among the different geographical areas in the 2000 study. In the 2000 study, growth was generally lowest at FB and highest at PG and BV. Mussel growth along the CPR was intermediate between the other geographic areas. Based on whole-animal weight growth rate, FB was significantly lower than the other three areas (in increasing order): $FB < CPR < BV = PG$ (Figure 1). Based on end-of-test tissue weights, each geographic area was significantly different from all others (in increasing order): $FB < CPR < PG < BV$ (Figure 2). Growth rates along the CPR were very similar across years for most mussel metrics. The largest difference occurred with end-of-test tissue weights, which increased more than twice as much in 1999 (76%) compared to 1998 (33%) and 2000 (32%).

Similar differences were found with respect to the PAH exposure metrics in the 2000 study. Based on end-of-test total PAH concentrations, PAH exposure was lowest along the CPR and highest at BV. Although the CPR and FB appeared to be a similar group and BV and PG appeared to be a group, there was some overlap among groups (Figure 3). Statistical groupings based on PAH content are more discriminating and suggest that BV and PG are distinguishable in terms of PAH exposure with Brownsville being the highest (Figure 4). These comparisons further support placing FB and the CPR in the low PAH exposure group. PAH accumulation in mussel tissues along the CPR was similar across years. As with mussel growth rates, the largest difference occurred in 1999 with a maximum observed tissue PAH concentration of 526 ug/kg-dry. This was about twice the amount measured in 1998 (313 ug/kg-dry) and in 2000 (249 ug/kg-dry). These differences could be attributed, at least in part, to the differences in temperature, growth rates, and end-of-test tissue weights in 1999.

Water temperatures in the 2000 study were significantly higher at PG and BV than at either the CPR or FB. Average temperatures were approximately 11.5 °C at PG and BV compared to only 10 °C at CPR and FB. This is understandable since PG and BV are the southernmost stations and because each is more enclosed. Although there was much overlap in the daily range of water temperatures, water temperature at the Cherry Point Reach was the most variable and least variable at Port Gamble. These data have potentially significant implications with respect to temperature as a stressor for herring egg development along the CPR and in other geographic areas of Puget Sound. Based on the results from the 2000 study alone, it could be concluded that temperature is not a significant stressor. Absolute temperatures were relatively low (CPR was the lowest) and the temperature range relatively small (CPR the highest). However, in the spring of 1998 (the El Nino year), temperatures along the CPR approached 17 °C and a 4°C maximum range in temperature was measured during a 4-hour period. In the summer of 1999, temperatures approached 18°C and a maximum range in temperature was measured during a 45-minute period. These data suggest that temperature is a potentially significant stressor for herring egg development along the CPR and demonstrates the value of in-situ monitoring and programmatic studies conducted over multiple years. If it can be assumed that the higher temperatures measured at PG and BV in 2000 are indicative of temperature differences that might have occurred during 1998 and 1999, even higher temperatures would have been measured in those areas in 1998 and 1999 compared to those measured at the CPR. Of all the factors measured in the 2000 study, temperature was more closely correlated with mussel growth and bioaccumulation of PAHs.

The most important findings of the 1998, 1999, and 2000 caged mussel studies were:

- For the CPR, mussels deployed at Mid Pier and Gulf Road accumulated the highest PAH concentrations on average between 1998 and 2000. This shows that these measurements were not an anomaly, but were based on the physical and chemical characteristics of the sites.
- PAH analyses that included alkylated homolog analyses demonstrated potentially different sources of PAHs at Mid Pier and Gulf Road.
- Other geographic areas within Puget Sound have significantly higher concentrations of PAHs and water temperatures than the CPR that could be affecting the survival and development of herring eggs.
- Even though potential stress from PAH and temperature exposure may be lower along the CPR these stressors have been identified as significant in the context of specific temporal and spatial scales.

These studies were successful in identifying PAH and temperature as potential stressors to herring egg development and characterizing PAH exposure and temperature at several locations covering a wide geographical area. These data can be used in conjunction with existing data and those collected later to clarify these and potential stressors to herring stocks and perhaps even rank their relative importance. Measurements made as part of the caged mussel studies may also help discriminate between “site” and “stock” effects. Results from three years of monitoring along the CPR have confirmed that bioavailable PAHs exhibit a patchy distribution. This is demonstrated by the consistently high PAH accumulation at Mid Pier and Gulf Road, associated with relatively low and highly variable water temperatures along the CPR. The relatively high amounts of PAHs accumulated by mussels in FB, PG, and BV suggests these areas may warrant additional characterization to determine if bioavailable PAHs are affecting herring egg development. The chemical fingerprinting through alkylated homolog analysis, in conjunction with the PAH concentration and content data, provide resource agencies multiple ways to assess PAH contamination in these different geographical areas and determine which areas, if any, require further characterization. The temperature data have provided a new perspective on potential adverse effects from a natural factor that is often inadequately characterized in monitoring studies. The use of caged mussels and *in-situ* temperature monitors provided valuable information in assessing exposure from point- and nonpoint stressors that could not have been collected using traditional methods.

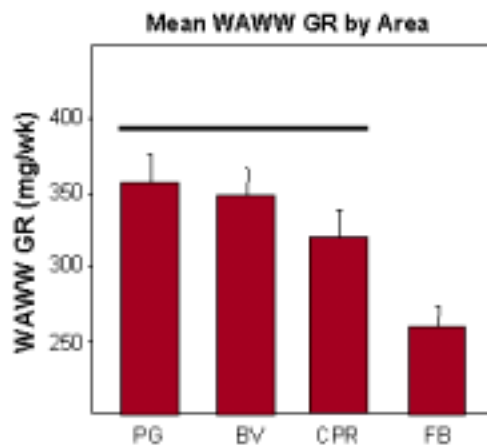


Figure 1. Mean WAWW growth rate (mg/wwk) by geographic area. Areas listed in order from highest to lowest. Underlined areas are statistically similar.

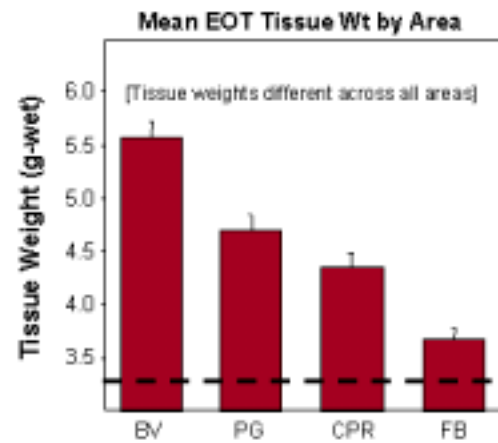


Figure 2. EOT tissue weight (g-wet) by geographic area. Dashed line represents initial tissue weight. Areas listed in order from highest to lowest. Underlined areas are statistically similar.

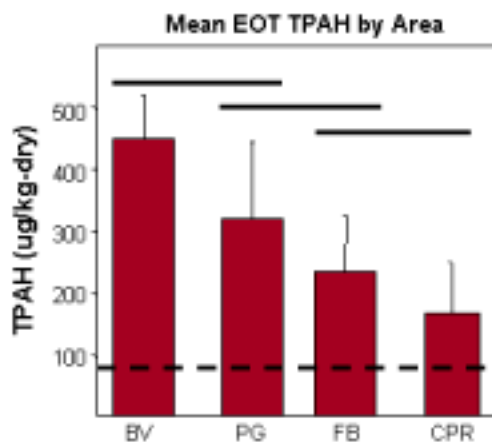


Figure 3. EOT tissue TPAH concentration (ug/kg-dry) by geographic area. Dashed line represents initial TPAH concentration. Areas listed in order from highest to lowest. Underlined areas are statistically similar.

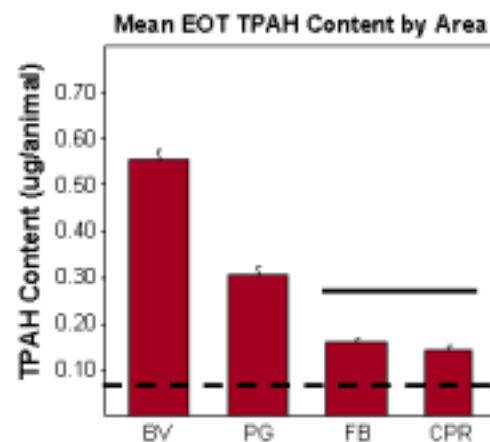


Figure 4. EOT tissue TPAH content (ug/animal) by geographic area. Dashed line represents initial TPAH content. Areas listed in order from highest to lowest. Underlined areas are statistically similar.